In the shadow of the condor: Invasive *Harmonia axyridis* found at very high altitude in the Chilean Andes

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Running Head: *Harmonia axyridis* at high altitude in the Andes

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Abstract. 1. *Harmonia axyridis* has invaded many regions of the world, with negative effects on local biodiversity, and thus it is of global concern for biological conservation. Recently it has invaded central Chile, one of the world's biodiversity hotspots, where the abundance and richness of ladybird species, particularly native species in agroecosystems, have declined following its arrival.

2. *Harmonia axyridis* is particularly abundant in spring in the valleys of central Chile, but there is a dramatic decline in its abundance during the hot summer months.

3. This study reports the occurrence of this invasive alien species in the summer at high altitudes (3578 m asl) in the Andes, which is the highest record worldwide. Individuals were observed on native cushion plants, in a unique environment rich in endemic species. *Harmonia axyridis* were active, reproducing and co-occurring with three other species of coccinellids.

4. The dispersal of *H. axyridis* and other coccinellids from the valleys to high altitudes could represent a mechanism to escape the adverse high temperatures during the summer, returning to the valleys in autumn. Our study highlights the need to study the impacts of invasive alien species across a range of habitats, including not only agricultural landscapes, but also extreme ecosystems.

Key words. Altitude, Coccinellidae, extreme ecosystems, harlequin ladybird, multi-coloured Asian ladybeetle, non-native species.

Introduction

The rate of translocation of species by humans beyond their native ranges is increasing and, for most taxa, there appears to be no indication of deceleration (Seebens *et al.*, 2017). The effects of alien species on biodiversity and ecosystem functioning are widely recognised (Simberloff *et al*., 2013). Invasive alien species, the subset of alien species that threaten biodiversity, society or the economy, are in part defined by their ability to spread rapidly within invaded regions. Some studies have suggested that natural habitats are resistant to invasion and evoke mechanisms related to the resident community structure and specifically species diversity (Lyons & Schwartz, 2001; Shea & Chesson, 2002).

One invasive alien species of global concern is *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), the harlequin or multicoloured Asian ladybeetle, that has invaded many regions of the world, with negative effects on local biodiversity, particularly native coccinellids (Alyokhin & Sewell, 2004; Harmon *et al.*, 2007; Brown *et al.*, 2011; Roy *et al*., 2012). In the last 25 years it has expanded its distribution to five continents, with Oceania (New Zealand) being added in 2016. Notably, this species reaches high population numbers in temperate regions of the northern and southern hemispheres, particularly in anthropogenic habitats (Roy *et al*., 2016).

In Chile, the first wild populations of *H. axyridis* were recorded in 2003 in the central zone, 100 km north of Santiago, but populations started to rise in 2010-2011, spreading rapidly throughout the country. More recently *H. axyridis* has reached very high abundance in crops and has also invaded natural habitats, such as the sclerophyllous matorral (Grez *et al*., 2016). This is of concern because central Chile is one of the world's 35 biodiversity hotspots, an important reservoir of biodiversity (Mittermeier *et al*., 2011). Also, this region has the highest number of endemic coccinellids in the country (Alaniz & Grez, unpublished data) which could be negatively affected by *H. axyridis*. Indeed there has been a decline in abundance and richness of ladybird species, particularly native species, within alfalfa crops following the arrival of *H. axyridis* (Grez *et al*., 2016).

In Chile it has been observed that *H. axyridis* reaches sustained high abundance in alfalfa crops during early spring. Noticeably, in summer its populations decline and it becomes almost absent within these crops until autumn, when it recolonizes for a short period of time before the onset of winter (Grez & Zaviezo, unpublished data). The phenology of *H. axyridis* within central Chile could be explained by the Mediterranean climate of this region, with wet and cold winters and hot and dry summers (Di Castri & Hajek, 1976), and by the low tolerance of *H. axyridis* to high temperatures (Benelli *et al*., 2015; Barahona-Segovia *et al*., 2016). The behavioural responses of *H. axyridis* under these unfavourable field conditions is unknown. One hypothesis is that during the summer it migrates towards places where temperatures are lower, for example high altitudes. This note reports the colonization by *H. axyridis* in the high Andes of central Chile, an extreme native habitat, during summer.

Study site

Two surveys were conducted during summer 2017 (29 January and 12 March), in the high Andes of central Chile, in the surroundings of Valle Nevado ski resort (UTM WGS84 19S 384386 E; 6313663 S), approximately 50 km east of Santiago (Appendix S1). This area has an alpine climate, with very cold winters (mean air temperature ~ 1.7°C, minimum of -15°C) and mild summers (mean air temperature ~ 6.8°C, and maximum 17°C) (Molina-Montenegro *et al*., 2006). In these two surveys, coccinellids were searched in an altitudinal gradient, from 2700 to 3600 m asl. In this area there are no trees or bushes, and the vegetation is very patchy, dominated by two native cushion plants (Apiaceae): *Azorella madreporica* Clos and *Laretia acaulis* (Cav.) Gill. et Hook (Fig. 1A). *Azorella madreporica* is a very flat and tightly knit cushion species, extending from 33°S to 50°S, and growing from above 3200 m asl in the Andes of central Chile (33°S) and close to sea level in its southern distribution (Hoffmann *et al*., 1998). *Laretia* *acaulis* is a hard resinous cushion plant native of the high Andes of Chile and Argentina, extending from 28°S to 35°S and most abundant between 2100 to 3100 m asl (Hoffmann *et al*., 1998). Cushion plants are one of the best-adapted growth forms in this habitat, generating more suitable micro-habitats for other plants and insects (Cavieres *et al*., 1998; Molina-Montenegro *et al*., 2009).

Results and discussion

In total, 37 adult *H. axyridis* (succinea form, the only recorded form in Chile so far; Grez & Zaviezo 2015) were found distributed from 2790 to 3578 m asl, all of them on patches of the cushion plants (Table 1). In lower altitude samples *H. axyridis* was absent, and the highest altitude where it was found coincides with the highest places with cushion plants in this area. Previous to this survey, from a citizen science observation reported through http://www.chinita-arlequin.uchile.cl/ (Grez & Zaviezo, 2015), one record at high altitudes was known in Chile (3086 m asl, 150 km north of the current records, Cordillera de Cuncumén; 372642 E 6460876 S) on *Eleocharis pseudoalbibracteata* Nes & Meyen ex Kunth. (Cyperaceae), another native plant. These records are the highest of *H. axyridis* worldwide. Other records of this species in high altitudes are from around 2500 m asl (Lesotho, southern Africa), and 1800 m asl (South Africa) (Stals 2010) in autumn-winter. In Europe it has been found in a range of altitudes up to 2280 m asl in Carinthia (Austria) (Roy *et al*., 2016). Additionally, *H. axyridis* is known to overwinter at moderate altitudes (e.g. on rocky mountains) in its native range (Wang *et al*., 2011). In contrast to these other high altitude records from around the world, data of this study were collected in summer. It has been reported that some ladybirds estivate at high altitudes (Stewart *et al*., 1967), but in our observations the majority of individuals were active and reproducing. Therefore, the high Andes would represent a refuge for *H. axyridis* during the summer, escaping from the high temperatures in the valley.

Together with *H. axyridis*, three other ladybirds were present: the native (and endemic) *Eriopis chilensis* Hofmann and two other non-native species, *Hippodamia convergens* (Guerin-Meneville) (a North American species first introduced to Chile in 1903, but only established in the early 90's) and *Hippodamia variegata* (Goeze) (a Eurasian species introduced from South Africa in 1967) (Fig. 1B - D). Additionally, the native *Adalia deficiens* Mulsant was found, but only at the lowest altitude samples (Table 1). Mainly larvae and adults of these species were observed, but also a few eggs and pupae. Previously, in summer 2003 and 2006, the first three species were also found in the same area of the present survey, associated with the same species of cushion plants, which were suggested to provide more suitable microclimatic conditions for the ladybirds in this habitat (Molina-Montenegro *et al*., 2006; 2009). Notably, in the previous studies at these high altitude sites *H. axyridis* was not observed, suggesting that this invasive alien species has only recently colonized the high Andes, considering that it had arrived in the adjacent valleys near Santiago in 2008.

Co-occurrence of *H. axyridis* with these other species may represent a threat to biodiversity as it has been documented in other regions, including the nearby valleys (Grez et al., 2016; Roy et al., 2016). It has been reported that *H. axyridis* is a strong competitor and intraguild predator of other coccinellids due to its larger size (*H. axyridis*: up to 8 mm; *H. convergens*: up to 6.5 mm; *H. variegata* and *E. chilensis* up to 6 mm; González, 2006) and strong physical and chemical defences (reviewed within Roy *et al*., 2016). The asymmetry of these interactions in favour of *H. axyridis*, coupled with its recent colonization of this unique habitat, could result in the displacement of the other species. Additionally, *H. axyridis* could contain high numbers of obligate parasitic microsporidia and Laboulbeniales fungi (Vilcinskas *et al*., 2013; Roy *et al.*, 2016), potentially impacting the health of the other species they interact with. Observations during the survey of mating attempts between a male *H. variegata* and a female *H. axyridis* may indicate that parasite transmission between species is plausible. The potential of *H. axyridis* of negatively impacting biodiversity in the high Andes is worthy of further study.

Apart from the species reported here, only two other ladybirds have been recorded at altitudes in excess of 3000 m: *Coccinella septempunctata* L. at up to 3475 m (Rice, 1992) and *Hippodamia quinquesignata* (Kirby) at up to 3354 m (Edwards, 1957), both in the USA. Also, in invertebrate surveys at the summit of Mauna Kea, Hawaii, *H. convergens* was found at a number of very high altitude sites up to 4226 m and *C. septempunctata* at a single site at the same altitude (Englund *et al*., 2010). Nevertheless, these insects were considered aeolian, i.e. not resident of the area where they were collected, but rather blown up from lower elevations (Englund *et al*., 2010).

In summary, this note provides evidence of the occurrence of the invasive alien species *H. axyridis* at high altitudes in the Andes near Santiago, in a unique environment rich in endemic species (Arroyo & Cavieres, 2013), which constitutes the highest report worldwide. This is unique not only because of the high altitude and associated extreme environment, but also because it highlights the way in which *H. axyridis* can survive adverse high temperatures during the summer within an invaded region through dispersal to elevated positions, a further mechanism explaining the success of this invading species. This report also highlights the importance of including landscape scale factors (i.e. altitude) into models predicting establishment, spread and ultimately impacts of invasive alien species to ensure that the diversity of habitats and associated niche opportunities for invading species are considered.

Conflict of interest

Authors have no conflict of interest.

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Supporting Information

Appendix S1. Map of central Chile and surveyed location.

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FIGURE CAPTIONS

Fig. 1 A) Surveyed habitat at Valle Nevado, Andes Mountains; B) *Harmonia axyridis* with a larva of *Hippodamia convergens*; C) male *Hippodamia variegata* mating with a female *Harmonia axyridis*; D) the native *Eriopis chilensis*;